The effects of zinc on enchytraeids: The Budel case study

J. Römbke
ECT Oekotoxikologie GmbH, Flörsheim, Germany

J. Notenboom & L. Posthuma
National Institute for Public Health and the Environment, (RIVM), Bilthoven, The Netherlands

Abstract

Species composition and abundance of enchytraeids are described along a transect of heavy metal contaminated soils around a former zinc smelter near Budel (The Netherlands). The results of this study can be summarised as follows:

- species composition and abundance are in a normal range in comparison to other forest sites in Central Europe;
- some plots, including but not only those close to the former zinc smelter, show rather low density values;
- the number of enchytraeids is variable among sample sites;
- there is a tendency for enchytraeid numbers to be correlated positively with the distance from the former zinc smelter plant and with zinc concentrations in soil;
- this distribution is probably influenced strongly by other factors than the zinc content alone.

The results are discussed in comparison with metal concentrations at the field sampling sites and with data from laboratory and semi-field tests with enchytraeids (performed with artificial and field soils). In addition, the findings are compared qualitatively with the enchytraeid coenosis that can be expected for such a site when taking the natural site parameters (especially soil factors) into consideration.

Keywords:
Enchytraeidae, ecotoxicological effects, The Netherlands, coniferous forest.

Introduction

The aim of the study was to determine the species composition and abundance of Enchytraeidae (Oligochaeta) along a gradient of heavy metal polluted soil sites in the neighbourhood of a former zinc smelter near Budel (The Netherlands) during 1995/1996. The results of this study are compared with data from other European coniferous forests sites, especially those which are also anthropogenically polluted by heavy metals. In parallel to the enchytraeid samples the concentration of zinc in the soil and in the tissue of two dominant species was measured. The field results were compared with laboratory and semi-field test data describing the effects of zinc on *Enchytraeus crypticus* and *Enchytraeus albidus*.

The sampling design of the field investigation is based on experience gained in a pilot study, in which samples were taken once in late 1995. In that study, six sites located along a gradient of ≈ 20 km length starting from the former zinc smelter were sampled using 10 replicates at each site. In 1996, samples were taken twice (in July and November) at 30 sites, covering approximately 30% of the original gradient, but with only one replicate.

The following questions are addressed:

- which species of Enchytraeidae are living in the coniferous forests of the Budel area and what abundance can be expected ?
- is there a correlation between the zinc concentrations at the sampling sites and the enchytraeid coenosis living there?
- what is the relation between the results of laboratory and semi-field tests with zinc and other metals using two *Enchytraeus* species as test organisms and the distribution pattern of enchytraeids in the field?

It should be noted that the investigation described here is only a small part of a larger project, in which, for example, bacteria, plants, collembolans, nematodes, and earthworms have been both tested in the laboratory and in soils from various sites (including the Budel gradient), with or without metal spiking or other pre-treatment. The project focused on the relevance of laboratory tests for predicting field effects of contaminants. Semi-field studies and field observations were made so that dominant factors which might cause irrelevant test results were investigated experimentally and empirically. Although not reported here, it will be possible to compare the effects of zinc on Enchytraeidae with the effects of this metal on other soil organisms. For further information see Notenboom & Posthuma (1994), Posthuma & Notenboom (1996), or Posthuma et al. (1998).

Material and methods

Sampling sites

The Budel zinc smelter area is located near the border between The Netherlands and Belgium (The Kempen). Smelter emissions (primarily zinc and cadmium) have occurred since 1892 till the 1980s. The area has been used for household dumps (early 20th century) and abandoned test plots for studying the effects of liming and nutrient enrichment on metal effects are present. Overall, the site can be identified as heterogenous with a dominant metal and pH gradient. The latter is probably caused by co-emissions of the smelting process or liming. The area surrounding the plant is used partly as a military training ground, partly for recreational purposes. The vegetation is mainly coniferous forest (*Pinus*), but grassland plots are not uncommon.

The soil is sandy with a clay content of less than 10 % (partly podzolic) and has an organic matter content of 1.9-5.5%. The humus type, mainly raw humus, is very heterogeneous on a small scale. There are strong differences between the individual sampling sites (e.g. due to truck traffic or military exercises). No correlation was found between the corresponding July and November moisture values (July: average 12.4%; minimum 3.5%, maximum 39.0%; November: average 21.1%; minimum: 2.5%, maximum 76.0%), whereas the pH was similar in both sampling periods (on average 3.6-3.7; minimum 2.5, maximum 5.0).

Enchytraeid and soil sampling

In a pilot study six sites were sampled (ten replicates each) within a distance of 20 km from the plant. In the main study, samples were taken at 30 sites twice (July and November 1996), but with just one replicate each. All thirty sampling sites were located on a gradient starting approximately 1 km north-east from the former zinc smelter plant and ending 6 km away. Sampling sites were numbered consecutively. The transect was not linear but partly also radial, and the lowest sample code numbers generally refer to sites closest to the plant. Samples were taken in areas of 1 m^2 each in coniferous (pine) forest area of different tree age. Samples were taken using a soil-corer (Ø 5.6 cm), differentiated into litter, 0-4 cm and 4-8 cm layers. The worms were sampled using a wet extraction method (approximately 96 h at a temperature of 10-15°C; Römbke 1995). Immediately after extraction the enchytraeids were identified alive according to Nielsen & Christensen (1959, 1961), Abrahamsen (1969) and O'Connor (1963). All adult worms were determined to the species level and the juveniles to the genus level. Their biomass was estimated based on individual measurements (Römbke 1989), applying a fresh to dry weight ratio of 0.149 (Axelsson et al. 1984). In autumn 1996, individual worms of two dominant species (*Marionina clavata*, *Cognettia sphagnetorum*) were fixed

in 70% alcohol after species identification and their zinc content was measured by AAS. In parallel to the worm samples, soil samples were taken for zinc analysis (three individual samples mixed up to one sample) and soil properties in a distance of less than 0.5 m from the worm samples. The data on total zinc concentrations in the soil are given in figure 1.

Tests methods

Since the main focus of this contribution is the field situation, the methods of the tests that were executed for laboratory/field comparisons are described only briefly (for details, see Dirven-Van Breemen et al. (1994) and Posthuma & Notenboom (1996)).

Test description: Laboratory

Design: Acute mortality and chronic reproduction tests with a duration of 4 weeks.

Species: *Enchytraeus crypticus* (partly *Enchytraeus albidus*).

Substrate: OECD Artificial Soil (pH = 6.0 ± 0.5; moisture 35%) or Budel field soil (pH 4.9-5.5, moisture ca. 20 %); in each case 7-10 g wet weight per test vessel containing 10-15 adult worms.

Parameter: Mortality of adults, number of juveniles (= juveniles per worm per week).

Conditions: Temperature: 17°C, food: oat flakes.

Evaluation: Results given in mg/kg zinc in soil (total and 0.01 M $CaCl_2$ exchange able); Normalisation of effect data on standard soil (i.e. set to 100% for the site with the lowest zinc concentration in soil).

Test description: Semi-field

Design: Chronic reproduction tests with a duration of 4 weeks.

Species: *Enchytraeus crypticus*.

Equipment: 10 cm long soil cores, placed in an experimental field plot (EFP), filled with soil from an uncontaminated site at Panheel, spiked with various zinc concentrations (nominal concentrations: 0-3200 mg Zn/kg dry soil, see Smit et al. (1997)).

Substrate: Panheel field soil (partly pH adjusted to 7.2, moisture variable); 50 adult worms were put initially in each soil core; the characteristics of Panheel and Budel gradient soils are similar, except for Panheel soil being uncontaminated.

Parameter: Number of juveniles (= juveniles per worm per week).

Conditions: Food: oat flakes (once at the beginning).

Evaluation: As in the laboratory tests.

Results

Field

An overview of enchytraeid abundance (ind/m²) in July and November 1996 in relation to the distance from the emission source is shown in Figure 2 (sum of the two soil layers). Despite the high variability at the individual sampling sites, the abundance of enchytraeids increases with increasing distance from the emission source as indicated by linear regression (for details see Römbke 1997).

Since the concentration of zinc was hypothesized as the main source of this distribution pattern, the abundance of enchytraeids (in upper (0-4 cm; left) and lower (4-8 cm; right) mineral soil samples in relation to the total zinc concentration was determined (Fig. 3). Again, the distribution pattern is characterized by a high variability. In total, the maximum numbers observed were 79.500 ind/m² in July (mean value: 9.900 ind/m²) and 73.400 ind/m² in November (mean value: 10.400 ind/m²). At many sites the July and November densities were quite similar, which is surprising when taking the overall variability into account. The most obvious differences were seen at two sites where in July very few enchytraeids and in November 31.000 and 73.400 worms were counted, respectively. The vast majority of sites

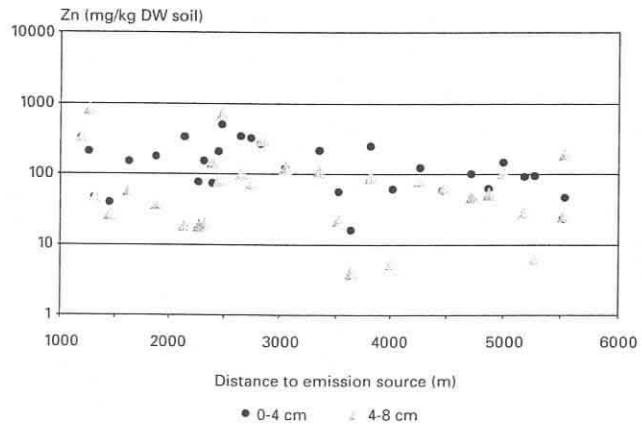

Fig. 1: Total zinc concentrations in the soil along the sampling transect northeast of Budel in two different soil layers (mg Zn/kg dry soil).

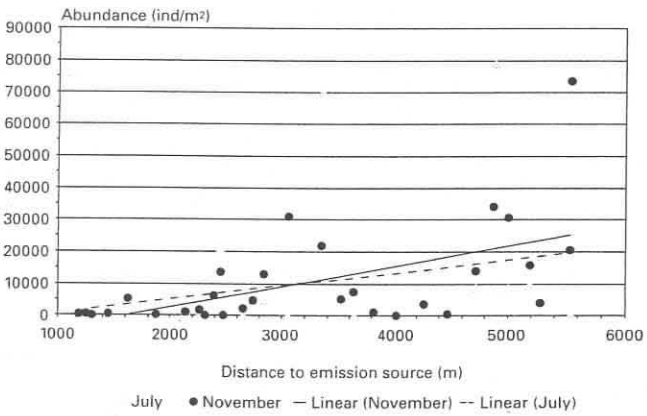

Fig. 2: Abundance of enchytraeids in individual mineral soil samples in relation to the distance to the emission source at the sampling dates (July and November 1996) (including regression trend lines).

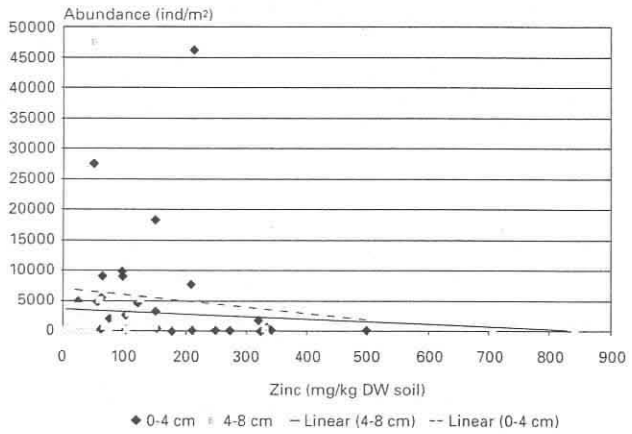

Fig. 3: Mean abundance of enchytraeids in upper (0-4 cm) and lower (4-8 cm) mineral soil samples in relation to the total zinc concentration (including trend lines); average of June and November data.

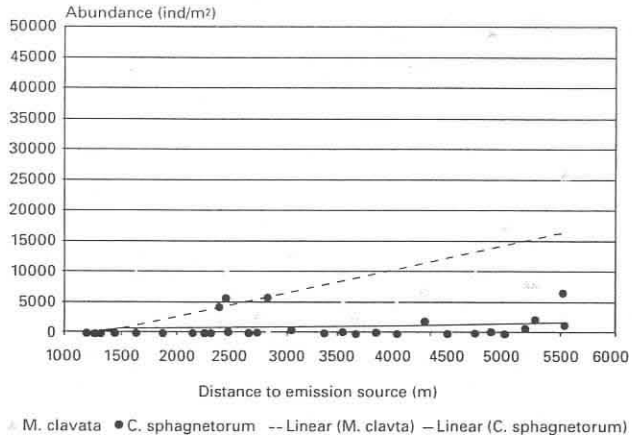

Fig. 4: Abundance (ind/m2) of *Marionina clavata* and *Cognettia sphagnetorum* in relation to the distance to the emission source (including trend lines).

had, however, densities that differed only by a factor of 2-3 at the two sampling dates. On average, the total biomass is 0.10 g DW/m² varying considerably between 0 and 0.83 g DW/m² at the individual sites.

In the pilot study, the ratio between enchytraeid numbers in the two soil layers showed a tendency to increase with the distance from the plant: Close to the former smelter, more worms preferred the deeper layer (ratio 1:3), whereas at the other sites the numbers were more or less equal or higher in the upper level. Such a tendency was not obvious in the 1996 data set. If there is a shift in the vertical distribution along the gradient, it is either time-dependent, or masked by the high variability between the various sites.

The species composition of enchytraeids in the Budel area is relatively simple. Nine species could be identified and just five of them occurred regularly (Tab. 1). The coenosis was dominated by *M. clavata* and *C. sphagnetorum* by number (Fig. 4) and biomass. These two species had different distribution patterns: the abundance of *M. clavata* increased clearly with increasing distance from the emission source whereas the abundance of *C. sphagnetorum* did not change along the

transect. Another three species (*A. "affinoides"*, *A. camerani*, *O. cambrensis*) are common inhabitants of coniferous forest sites in the Budel area. The ecological requirements and biogeography of the most important species are described by Beylich et al. (1995), Graefe (1993), Healy (1980) and Römbke (1992). Despite very low absolute numbers, it is interesting that *E. norvegicus* is only found at sites 1 and 7 since species of this genus are considered to be indicators of disturbed sites (Graefe 1997). Overall, the species composition results indicate different sensitivities of the various species towards zinc.

Genus and species	Dominance	Remarks
Achaeta abulba	-	Only in one replicate far away from the plant
Achaeta "affinoides"	6.5%	Taxonomically not described yet; widely distributed in European acid soils
Achaeta camerani	0.1%	Widely distributed in European acid soils
Achaeta DKL	-	Taxonomically not identified, since only juveniles were found (in just one replicate) far away from the plant
Cognettia sphagnetorum	17.8%	Fragmenting species which is very widely distributed in acid soils of the Northern Hemisphere
Enchytraeus norvegicus	3.2%	The only species of this genus known from acid soils; widely distributed in Central and Northern Europe but rarely in high numbers
Fridericia perrieri	-	Found only in one replicate far away from the plant
Marionina clavata	69.9%	Widely distributed in very acid soils
Oconnorella cambrensis	2.5%	Widely distributed in acid soils

Table 1: Species composition of enchytraeids at the Budel site.

Due to the often relatively low numbers, the variability of the occurrence of the individual species among sampling sites is high. However, there is a tendency for the number of species found in a sample to increase with decreasing zinc concentration.

The measurement of zinc in individuals of the species *M. clavata* as shown in figure 5 revealed two major findings concerning the internal zinc concentration of the worms (the results of *C. sphagnetorum* did not differ strongly):

- the absolute values are very high (usually between 500 and 4000 mg Zn/kg DW of body weight; in a few cases concentrations of 9488 or 14872 mg Zn/kg DW of body weight were found);
- the internal concentration is nearly identical over a wide range of soil concentrations.

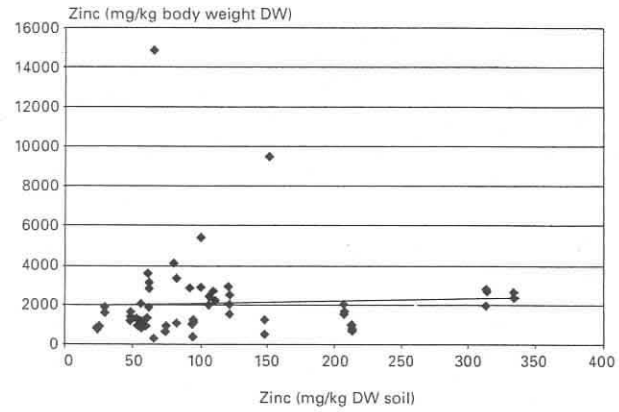

Fig. 5: Internal concentration of individuals of the species *Marionina clavata* in relation to the total zinc concentration in the soil (including trend line).

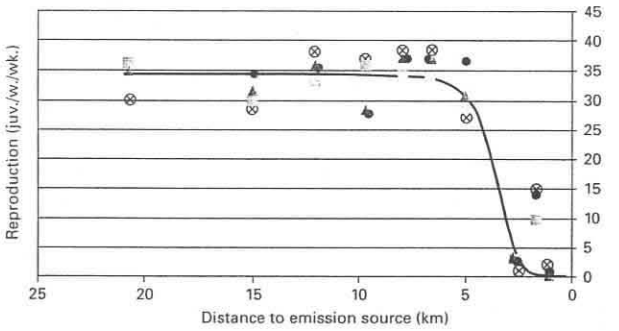

Fig. 6: Reproduction of *Enchytraeus crypticus* in pH-adjusted Budel field soil samples taken at different distances from the plant, when reared in laboratory conditions. Replicates are indicated by different symbols.

Laboratory and semi-field

In table 2, the results of various laboratory tests with *E. crypticus* using artificial (OECD 1984), Panheel soil (fresh and aged) as well as Budel soil are shown. The EC50 values based on total concentrations ranged from 205 to 844 mg total Zn/kg soil, or (equivalently) from 57 to 149 mg exchangeable zinc/kg soil. The coefficient of variation of EC50 values among tests was similar when using total concentrations or the assumed bioavailable fraction (= 0.01 M $CaCl_2$ extractable) as a measure of exposure (both approximately 60-70%). In any case the concentrations (roughly between 10 and 1000 mg/kg Fig. 1) measured in the field are in the same order of magnitude as the effect concentrations found in the single-species tests. This is highlighted when looking at the reproduction of *E. crypticus* in pH-adjusted Budel field soils taken at different distances to the plant (replicates are indicated by different symbols; Fig. 6). Approximately 5 km from the plant the soil becomes highly inhibiting for reproduction as shown by the decreasing number of juveniles. It should be noted that *E. crypticus* showed roughly the same sensitivity as *E. albidus* but was clearly more sensitive to the metal than the lumbricid earthworm *Eisenia andrei* (Posthuma & Notenboom 1996).

Test Soil	Total Zn	0.01 M $CaCl_2$ extractable
OECD soil	248	61
S-F soil (fresh)	262	149
S-F soil (aged)	844	102
Budel field	205	57
Average	319	93
Coeff. Variance	68	64

Table 2: Effect levels (EC50) for *E. crypticus* after testing their sensitivity to zinc in different soils (mg Zn/kg dry soil); S–F = Semi-field.

Discussion

Species abundance and diversity in Dutch coniferous forests

In The Netherlands, very few taxonomically orientated investigations have been carried out in coniferous forests (Arnhem, Hoge Veluwe De Pollen) since the early Sixties (De Gunst 1965). Average density, based on annual samplings, are not available and therefore, some data from Central Europe and Scandinavia are summarised in table 3 (e.g. Didden 1993; Petersen & Luxton 1982; Römbke et al. 1997). The high variability of these data indicate that coniferous forests contain varying enchytraeid communities. Only very rough conclusions can be drawn: the abundance is usually moderate (approx. 50.000 ind/m^2), sometimes very low but never exceptionally high (> 100.000 ind/m^2) and the same is true for the biomass. The number of species is usually lower than 5 (often only 1-2) and never higher than 14.

The four species *A. camerani*, *C. sphagnetorum*, *O. cambrensis* and *M. clavata* found at Budel seem to be typical for coniferous forests, as assessed from data collected elsewhere in Europe. For example, Beylich et al. (1995) reported *M. clavata*, *Achaeta brevivasa*, *C. sphagnetorum* and *A. camerani* from a spruce stand in Schleswig-Holstein (Northern Germany). Römbke et al. (1997) mention *C. sphagnetorum* and *M. clavata* as the most frequent species in Central European coniferous forests. Additionally, the occurrence of other species of the genus *Achaeta*, which are often found at such forest sites, is verified at Budel. Only the occurrence of *E. norvegicus* in the Budel gradient was not expected because this species prefers slightly higher pH values than the other species which are more acidophilous.

In comparison to these literature values, it seems that the enchytraeid coenosis at Budel can be classified as normal (table 3), but it should be remembered that the Budel values are based on only two samplings.

Parameter and Biotope	Mean ± Standard Deviation	Minimum	Maximum
Density Ind./m² (* 10³)			
Forests with Raw Humus	53.5 ± 55.3	4	134
Budel	10.2	0	73
Biomass g dry weight/m²			
Forests with Raw Humus	0.82 ± 1.25	0.17	2.69
Budel	0.10	0	0.83
Number of Species			
Forests with Raw Humus	4.3 ± 0.6	1	14
Budel	6	0	5

Table 3: Abundance, biomass and number of species of Central European and Scandinavian coniferous forests with raw humus. All data except from Budel are based on average annual values taken from the literature.

Effects of anthropogenic factors on enchytraeids

It was hypothesised that the distribution of enchytraeid species in the field would be affected by zinc. This expectation was based on the observation that laboratory tests with *E. crypticus* showed an almost complete inhibition of reproduction, and hence, a large reduction of population density in the field could be expected at sites within approx. 5 km from the plant. This expectation was not confirmed by the field data on enchytraeid densities. The densities at depths of 0-4 and 4-8 cm varied but with a tendency to decrease in the 0-4 cm layer towards the plant and with increasing soil zinc concentrations. Apparently, zinc is not the sole factor affecting density since that would cause a sharp sigmoidal density decrease along the gradient. Alternative explanations for the observed Budel gradient enchytraeids coenoses are:

1. Heterogeneity – this was observed for example for pH, a factor that showed the highest values in the samples nearest to the factory (due to co-emissions of neutralising agents with past zinc manufacturing) but was also variable.
2. Indirect effects – although not determined, this is a factor absent in single-species laboratory tests; the factor might, however, be important, since within the enchytraeid coenosis, or outside (competitors, predators) changes in dominance may occur as an indirect effect of zinc or other soil characteristics.
3. Bioavailability in relation to pH – the special case in Budel might be that the dominant gradient in response to total zinc is largely counteracted by an opposite effect of pH, via the reduction of zinc exposure of enchytraeids at the increased pH-values close to the plant.

Overall, it is concluded that these major causes cannot be disentangled on the basis of the available data. However, it can be concluded that the distribution of enchytraeids in a field gradient is more complex than in laboratory toxicity tests. This means that ecological extrapolation (what does laboratory toxicity data mean

for field density of an exposed species) should be executed with care. However, macro-scale extrapolation in the framework of environmental management (based on a large sample of laboratory-toxicity data across species to derive "ecotoxicological risk limits" for generic use in environmental legislation (e.g., Crommentuijn et al., 1997)), need not be invalidated by this observation. That is, the risk limits derived using toxicity data that are not fully field-representative, can still serve the policy purpose for which they are intended as they correctly distinguish between acceptable and unacceptable risks.

The most important studies for comparison with the Budel study is a field investigation performed by Bengtsson & Rundgren (1982). These authors extracted Enchytraeidae from soil in mature spruce forests at various distances from a brass mill in Gusum (Sweden). The concentrations of zinc, copper and lead increased near the mill, where species number (from 8 to 1-2) and abundance (from ≈ 17.000 to less than 1000 ind/m^2) of these worms was significantly reduced. Additionally, the vertical distribution was different at sites close to the mill compared to unpolluted sites. The authors reported a low variability at Gusum, which is different from the findings reported here.

Comparison between laboratory/semi-field tests and field results at Budel

In various laboratory experiments the internal concentration of zinc in enchytraeids has been measured, showing results in the same order of magnitude:

- max. between 558 mg Zn/kg DW of body weight (Peijnenburg et al. 1999) and +/- 700 mg Zn/kg DW of body weight (Posthuma et al. 1997) for *Enchytraeus crypticus*;
- max. between 1520 mg Zn/kg DW of body weight (Rüther & Greven 1990) for *Enchytraeus buchholzi*;
- max. +/- 2500 mg Zn/kg DW of body weight (Lock et al. 2000) for *Enchytraeus albidus*;
- max. +/- 1500 mg Zn/kg DW of body weight (Sjögren et al. 1995) for *Cognettia sphagnetorum*.

In *M. clavata* and *C. sphagnetorum* concentrations higher than 2500 mg/kg Zn DW were found in approximately 70% of all individuals. The difference between the literature values and the higher values in the Budel soil samples might be caused by methodology (e.g. the storage of the Budel animals in alcohol before metal detection). However, the overall conclusions are not influenced. Firstly, the body concentrations of zinc in enchytraeids in the field are at least as high as in laboratory experiments. Secondly, these results seem to indicate that enchytraeids are able to regulate zinc in soil concentrations encountered in the Budel gradient. The same conclusion was found when investigating the zinc bioavailability for *Enchytraeus*

crypticus in 20 Dutch contaminated field soils, without alcohol storage prior to analysis (Peijnenburg et al. 1999).

During the last ten years, the effects of various metals on Enchytraeidae were studied at the RIVM (Dirven-Van Breemen et al. 1994; Notenboom & Posthuma 1994; Posthuma & Notenboom 1996). Tests with standard (artificial) soils as well as spiked field soils from an uncontaminated site (Panheel) and from the Budel area were performed, using different *Enchytraeus* species as test organisms. The test endpoint was mainly reproduction. These studies can be briefly summarised as follows:

- the reproducibility of the data gained in laboratory tests with the same soil and exposure condition is very high;
- differences in results when using different soils can partly be explained by physico-chemical factors, quantitatively or qualitatively (e.g. bioavailability differences between soils and ageing effects respectively);
- effect levels of laboratory and semi-field tests are comparable for the same test endpoint (reproduction);
- in the contaminated field gradient soils from Budel, toxicity is largely caused by zinc (and not by co-occurring metals like cadmium).

Based on these results, the following conclusions can be drawn concerning the field work:

- changes in species composition and species densities cannot be explained by zinc concentrations alone;
- an additional factor such as pH has probably influenced the impact of zinc on the enchytraeid biocoenosis in an indirect way (via availability), other factors may influence the biocoenosis directly;
- low species and density numbers as well as the high variability and co-variation of soil factors have impeded identification of major trends and likely causes expected from laboratory toxicity data. A small range of laboratory toxicity data for zinc on some enchytraeid species from congeneric test species does not provide an exact ecological prediction of a field biocoenosis of enchytraeids in a heterogeneous gradient with zinc
- despite the former conclusion, the collected data do not invalidate the derivation method for risk limits of zinc as currently applied in the Netherlands and elsewhere.

Acknowledgements

Several colleagues from the RIVM have participated to this study (especially D. de Zwart, A.-J. Folkerts, G. P. C. Zweers, J. van der Velde, R. Baerselman and E. M. Dirven-van Breemen); their contributions are greatly appreciated. In addition, two anonymous reviewers made valuable contributions to a former version of this manuscript.

References

Abrahamsen, G., 1969: *Enchytraeus norvegicus* sp.nov.: A new species of Enchyraeidae (Oligochaeta) from Norway. - Nytt Mag. Zool. 17: 161-164.

Axelsson, B., U. Lohm & T. Persson, 1984: Enchytraeids, lumbricids and soil arthropods in a northern deciduous woodland - a quantitative study. - Holarctic Ecol. 7: 91-103.

Bengtsson, G., & S. Rundgren, 1982: Population density and species number of enchytraeids in coniferous forest soils polluted by a brass mill. - Pedobiologia 24: 211-218.

Beylich, A., H.-C. Fründ & U. Graefe, 1995: Environmental Monitoring of Ecosystems and Bioindication by Means of Decomposer Communities. - Newsletter on Enchytraeidae 4: 25-34.

Crommentuijn, T., M. D. Polder & E. J. van de Plassche, 1997: Maximum Permissible Concentrations and Negligible Concentrations for metals, taking background concentrations into account. - Bilthoven, The Netherlands: National Institute of Public Health and the Environment; RIVM-report no. 601501001.

De Gunst, J. H., 1965: Enchytraeidae of the Netherlands (Annelida; Oligochaeta). - Beaufortia 13: 5-12.

Didden, W., 1993: Ecology of terrestrial Enchytraeidae. - Pedobiologia 37: 2-29.

Dirven-van Breemen, E., R. Baerselman & J. Notenboom, 1994: Onderzoek naar de Geschiktheid van de Potwormsoorten Enchytraeus albidus en *Enchytraeus crypticus* (Oligochaeta, Annelida) in Bodemecotoxicologisch Onderzoek. - RIVM Rapport Nr. 719102025. 46 pp. (In Dutch).

Graefe, U., 1993: Die Gliederung von Zersetzergesellschaften für die standortsökologische Ansprache. - Mitt. Deutschen Bodenkundl. Ges. 69: 95-98. (In German).

Graefe, U., 1997: Bodenorganismen als Indikatoren des biologischen Bodenzustands. - Mitt. Deutschen Bodenkundl. Ges. 85 II: 687-690. (In German).

Healy, B., 1980: Distribution of terrestrial Enchytraeidae in Ireland. - Pedobiologia 20: 159-175.

Lock, K., C. R. Janssen & W. M. de Coen, 2000: Multivariate Test Designs to Assess the Influence of Zinc and Cadmium Bioavailability in Soils on the Toxicity to *Enchytraeus albidus*. - Environ. Toxicol. Chem. 19: 2666-2671.

Nielsen, C. O., & B. Christensen, 1959: The Enchytraeidae, critical revision and taxonomy of European species. - Natura Jutlandica 8-9, 1-160.

Nielsen, C. O., & B. Christensen, 1961: The Enchytraeidae, critical revision and taxonomy of European species. Supplement 1. - Natura Jutlandica 10: 1-23.

Notenboom, J., & L. Posthuma, 1994: Validatie Toxiciteitsgegevens en Risicogrenzen Bodem: Voortgangsrapportage 1993. - RIVM-Rapportnr. 719102029, 70 S. (In Dutch).

O'Connor, F. B., 1963: *Marionina cambrensis* sp.n.: a new enchytraeid worm (Oligochaeta) from North Wales. - Ann. Mag. Nat. Hist. 6: 761-766.

OECD (Organisation for Economic Co-operation and Development) 1984: OECD-Guideline for Testing of Chemicals No. 207. Earthworm Acute Toxicity Test. Paris.

Peijnenburg, W. J. G. M., L. Posthuma, P. G. P. C. Zweers, R. Baerselman, A. C. de Groot, R. P. M. van Veen & D. T. Jager, 1999: Prediction of Metal Bioavailability in Dutch Field Soils for the Oligochaete *Enchytraeus crypticus*. - Ecotoxicol. Environ. Saf. 43: 170-186.

Petersen, H., & M. Luxton, 1982: A comparative analysis of soil fauna populations and their role in decomposition processes. - Oikos 39: 287-388.

Posthuma, L., & J. Notenboom, 1996: Toxic effects of heavy metals in three worm species (*Eisenia andrei, Enchytraeus crypticus* and *Enchytraeus albidus*) exposed in artificially contaminated soil substrates and contaminated field soils. - RIVM Report No. 719102048, Bilthoven, 78 pp.

Posthuma, L., R. Baerselman, R. P. M. van Veen & E. M. Dirven-van Breemen, 1997: Single and joint toxic effects of copper and zinc on reproduction of *Enchytraeus crypticus* in relation to sorption of metals in soils. – Ecotoxicol. Environ. Saf. 38: 108-121.

Posthuma, L., C. A. M. van Gestel, C. E. Smit, D. J. Bakker & J. W. Vonk, 1998: Validation of toxicity data and risk limits for soils: final report. - RIVM Report No. 607505004, Bilthoven, 230 pp.

Römbke, J., 1989: Zur Biologie eines Buchenwaldbodens. 12. Die Enchytraeen. - Carolinea 47: 55-92. (In German).

Römbke, J., 1992: Contributions to the biogeography of some species of terrestrial Enchytraeidae (Oligochaeta: Annelida). - Soil Biol. Biochem. 24: 1283-1290.

Römbke, J., 1995: Enchytraeen (Oligochaeta) als Bioindikator. - UWSF - Z. Umweltchem. Ökotox. 7: 246-249. (In German).

Römbke, J., 1997: Field sampling of Enchytraeidae at Budel (The Netherlands). - Report for the RIVM, 33 pp.

Römbke, J., L. Beck, B. Förster, H.-C. Fründ, F. Horak, A. Ruf, C. Rosciczewski, M. Scheurig & S. Woas, 1997: Boden als Lebensraum für Bodenorganismen und bodenbiologi-sche Standortklassifikation - Literaturstudie. - Texte und Berichte zum Bodenschutz 4/97. Landesanstalt für Umweltschutz Baden-Württemberg (Karlsruhe). 390 S. + Anhang. (In German).

Rüther, U., & H. Greven, 1990: The effect of heavy metals on Enchytraeids. 1. Uptake from an artificial substrate and influence on food preference. - Acta Biol. Benrodensis 2: 125-131.

Sjögren, M., A. Augustsson & S. Rundgren, 1995: Dispersal and fragmentation of the enchytraeid *Cognettia sphagnetorum* in metal polluted soil. – Pedobiologia 39: 207-218.

Smit, C. E., P. van Beelen & C. A. M. van Gestel, 1997: Development of zinc bioavailability and toxicity for the springtail Folsomia candida in an experimentally contaminated field plot. - Environ. Pollut. 98: 73-80.